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ANTIPLASMODIAL STUDIES ON THE ETHYL ACETATE FRACTION OF THE STEM BARK EXTRACT OF *Uapaca togoensis* PAX. (EUPHORBIACEAE) IN MICE

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ABSTRACT

Uapacatogoensis of family Euphorbiaceae, has been used folklorically for the treatment of various ailments including pneumonia, cough, fever, rheumatism, vomiting and epilepsy. The ethyl acetate fraction of the stem bark was analyzed for antiplasmodial activity in mice. Acute toxicity of the extract was studied Lorke's method. Suppressiveness, curative and prophylactic effect was studied in chloroquine-sensitive *Plasmodium berghei* NK 65 infected mice. Five groups, of six mice in each group were used. Group 1 was administered with 0.2ml distilled water only; groups 2, 3 and 4 were administered with 250, 500, and 1000 mg/kg ethyl acetate fraction of *Uapacatogoensis* respectively, while group 5 was administered with 5mg chloroquine/kg body weight. The doses were all administered orally. All doses of the extract produced significant, dose dependent, chemo suppressive activity against the parasite in the suppressive, curative and prophylactic tests. This was comparable to the group treated with chloroquine. The extract also prolonged the mean survival time of treated mice compared to the untreated group. The oral median lethal dose (LD₅₀) of the ethyl acetate fraction in mice was greater than 5000mg/kg body weight. The results of this study showed that the ethyl acetate fraction of the stem bark is safe and has antiplasmodial activity.

Key words: Acute toxicity, Antiplasmodial, Ethyl acetate fraction, *Uapacatogoensis*

INTRODUCTION

Malaria is an infectious disease that has continued to cause mortality and morbidity in the tropics and subtropics (Igwe et al., 2012). Malaria is transmitted to people through the bites of infected female Anopheles mosquitoes, blood transfusion, organ transplantation, shared use of needles and syringes that are contaminated with blood or from a mother to her foetus before or during delivery (WHO, 2015). There are five species of *Plasmodium* that cause malaria in humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium knowlesi* (Collins, 2012; Tcherniuk et al., 2015). Malaria caused by *P. falciparum* is more deadly than that caused by the other species of the malaria parasite (Sarkar, 2009). In Africa, most deaths occur among infants, children, pregnant women, and patients with HIV/AIDS, as well as non-immune migrants, mobile populations and travellers (WHO, 2015).

Medicinal plants since time immemorial have been used as a source of medicine (Horeau and Da Silva, 1999). The two main antimalarial drugs in use today were derived from plants that had been used traditionally over the centuries in the treatment of

malaria and other diseases – quinine from the bark of Cinchona tree and artemisinin from *Artemisia annua* (Basco et al., 1994, Addis et al., 2001 and Odugbemi et al., 2007). In Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of the children with high fevers, resulting from malaria, is the use of herbal medicines at home (WHO, 2002). The increasing resistance of malaria to orthodox antimalarial drugs and adverse effects associated with the drugs is one of the greatest challenges facing malarial control today (Mendis et al., 2001; Iwuanyanwua et al., 2012). This situation coupled with the high cost and/or inaccessibility to effective antimalarial drugs has stimulated research into the development of newer and more effective alternatives from the plant kingdom which could be more affordable.

Uapacatogoensis (Pax) family Euphorbiaceae is a tree about 13 m high with a short bole, usually without stilt-roots on dry sites, but may be stilted in damp localities, of savanna regions from Senegal to western Cameroon, and in eastern Cameroon to Central African Republic. Sometimes in Sierra Leone it forms nearly pure stands (Burkhill, 1994).

Ethnobotanical/pharmacological studies revealed that *Uapaca togoensis* has a wide range of activities. *U. togoensis* is used for treating female infertility and as a restorative wash against fatigue and for making charcoal (Irvine, 1948). The plant has been used folklorically for the treatment of various ailments including pneumonia, cough, fever, rheumatism, vomiting and epilepsy (Kone *et al.*, 2007). Extracts from different parts of the plant have been used in the treatment of skin diseases (Ajibesin, 2012). Compounds from the fruits of *Uapaca togoensis*, have been isolated and have shown good anti cancer activity against nine drug sensitive and multidrug-resistant cancer cell lines (Kuate *et al.*, 2015). The antifungal and antimicrobial activities of the plant has also been reported (Omachi *et al.*, 2015).

With this view, the present study was executed to investigate the antiplasmodial activity of the ethyl acetate fraction of stem bark extract of *Uapaca togoensis* in *Plasmodium berghei* infected mice in order to provide scientific evidence for its use in the therapeutic management of malaria.

MATERIALS AND METHODS

Experimental materials

Plant material

The stem bark of *Uapaca togoensis* were collected in February 2013 from Edumoga district, Okpokwu Local Government Area, Benue state. The plant was identified and authenticated by a botanist in the Department of Biological Sciences, Ahmadu Bello University, Zaria. A voucher specimen with number 1279 was deposited in the herbarium for future reference.

Animals

Swiss albino mice of both sexes weighing 18-22g were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. Animals were maintained under standard environmental conditions with access to standard diet and water *ad libitum*.

METHODS

Preparation of plant extract

The freshly collected stem bark of the plant *Uapaca togoensis* was air-dried under shade for 2 weeks. The dried stem bark were pulverized and reduced to powder using a mortar and pestle. The powdered stem bark (2 kg) was exhaustively subjected to cold maceration with 70%v/v methanol for 72 hours with intermittent agitation. The extract was filtered with the aid of a filter paper (Whatman No. 3) and the filtrate was concentrated to dryness at room temperature. The crude methanol extract (50g) was further partitioned with 200ml of ethyl acetate. The resultant filtrate was concentrated to dryness and used for the study.

The parasite *Plasmodium berghei*

Plasmodium berghei was obtained from the Department of Microbiology, National Medical Research Institute Yaba, Lagos. The parasite was maintained by continuous re-infestation intraperitoneally in mice every 4 days (Adzu *et al.*, 2007). Before commencing the study, one of the

infected mice was isolated and observed to reproduce signs and symptoms of malaria similar to those signs and symptoms exhibited by humans (Ramakrishnan *et al.*, 2012). Each mouse used in the experiment was inoculated with 0.2ml of infected blood containing about 1×10^7 *plasmodium berghei* parasitized red blood. This was prepared by determining both the percentage parasitaemia and the erythrocytes count of the donor mouse and diluting them with normal saline in proportion indicated by both determinations (Okonkon *et al.*, 2010).

Acute Toxicity Studies

The oral median lethal dose of the ethyl acetate fraction of *Uapaca togoensis* (EAFUT) was determined in mice using the method of Lorke (1983). Animals were fasted overnight prior to administration of the fraction. In the initial phase, three (3) groups each consisting of three mice was treated with the ethyl acetate fraction of the plant at doses of 10, 100 and 1000mg/kg body weight orally and observed for signs of toxicity for 4 hours. After 24 hours, the second phase was initiated. In the second phase four groups each containing one animal was administered with four more specific doses of the fraction in geometrically increasing order (1,200mg/kg, 1,600 mg/kg, 2,900 mg/kg and 5,000mg/kg). The animals were then observed for signs of toxicity and mortality for another 24 hours. The LD₅₀ value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1 and 1/1) respectively.

Preliminary Phytochemical Screening

Phytochemical analysis of the fraction was conducted using standard qualitative method as described by Trease and Evans (2002).

Evaluation of Suppressive Activity in Early Infection (4-day test).

The method described by Peters and Robinson (1992) was employed. *Plasmodium berghei* (chloroquine sensitive strain) was used to infect Swiss albino mice for a 4 day suppressive test. A total of thirty mice were used for this study. Each mouse was given standard intra-peritoneal inoculum of $1.0 \times 10^7 P. berghei$ parasites with the aid of a 1 ml disposable syringe. The animals were divided into five groups of six mice each. Different doses of the ethyl acetate fraction (250, 500, 1000 mg/kg/day) were administered orally to the groups. Chloroquine 5 mg/kg/day was given as positive control and 0.2 ml of distilled water as negative control for four consecutive days (D0 to D3). On the fifth day (D4), thin blood smears were prepared and blood films were fixed with methanol. The blood films were stained with Giemsa, and then microscopically examined with 100-x magnification. The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected controls with those of treated mice. The slides were examined under the microscope and parasitaemia determined by counting the number of parasitized erythrocytes in four fields.

Average percentage chemo suppression was calculated as $= (A-B)/A * 100\%$

Where A = Average parasitaemia in negative control

B = Average parasitaemia in positive groups

Evaluation of Curative Activity in Established Infection (Rane's test).

The method described by Ryley and Peters (1970) was employed. On the first day (day 0) of the study, thirty mice were inoculated intraperitoneally with 0.2ml standard inoculums containing approximately 1×10^7 *Plasmodium berghei* erythrocytes. Seventy two hours later (day 3), the mice were randomly divided into five groups containing six mice each of both sexes. Group one was administered 0.2ml of distilled water orally. Groups 2-4 received graded doses of the fraction (250mg/kg, 500mg/kg and 1000mg/kg) while group 5 received 5mg/kg of chloroquine (positive control). The fraction and drugs were administered orally daily for 4 days (day 4 -7). On day seven, each mouse was tail bled and a thin blood film was made on a microscope slide. The films were fixed in absolute methanol, stained with Giemsa and examined microscopically to determine the parasitaemia level. After the seventh day, the animals were fed *ad libitum* and observed for 29 days (day 0-28). Percentage chemo-suppression relative to the negative control was determined for each dose as previously described. Any death that occurred during this period (day 0-28) was noted and the mean survival time (MST) was calculated (Saiduet *al.*, 2000; Adzu and Salawu, 2009; Alliet *al.*, 2011).

$MST = (\text{Number of days survived}) / (\text{Total number of days})$

Evaluation of Prophylactic (repository) Activity

The prophylactic activity of the extract was investigated using the method described by Peters (1965). 30 mice of both sexes were randomly divided into five groups each containing six mice. Group 1 was administered with 0.2ml distilled water, groups 2, 3 and 4 were administered with 250, 500 and 1000 mg fraction /kg body weight orally respectively, while group 5 was administered with 1.2mg pyrimethamine/kg body weight orally daily. Treatment continued daily for three days (D_1 - D_3) and mice were all infected with the parasite on the fourth day (D_4). Thin blood films were prepared from each mouse 72hours (D_7) post treatment and mean parasitaemia in each group determined microscopically. Thin blood films stained with Giemsa stain were prepared from tail blood of each mouse and percentage chemo suppression calculated according to the formula stated previously.

Statistical Analysis

Data were expressed as mean \pm standard error of mean (SEM). Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by

Dunnett t-test. Results were considered to be significant at $p < 0.05$.

RESULTS

Preliminary Phytochemical Screening

The ethyl acetate fraction of the stem bark of *Uapaca togoensis* comprised of glycosides, phenols, triterpenes, steroids, alkaloids, tannins, carbohydrates, saponins and flavonoids (Table 1).

Acute Toxicity Studies

The median oral lethal dose of the stem bark extract of *Uapacatogoensis* in mice was found to be greater than 5000mg/kg body weight. There was a remarkable increase in activity in the mice within the first four hours. Nomortality occurred within the observation period of 48 hours.

Antiplasmodial Activity

Effect of Suppressive Activity of the Ethyl Acetate Fraction of the Stem Bark *U. togoensis*

The administration of the fraction resulted in a significant dose-dependent decrease in parasite counts. The average parasite percentage chemo suppression recorded was 39.1, 53.9 and 72.7% at 250, 500 and 1000 mg extract/kg body weight, respectively, while administration of the standard reference drug (Chloroquine 5mg/kg body weight) produced 98% chemo suppression (Table 2).

Schizonticidal Activity of the Ethyl Acetate Fraction of the Stem Bark of *U. togoensis*

The administration of the fraction also resulted in significant and dose dependent decrease in parasite counts in the treated groups when compared to the control group (Table 3). The mean parasite count was 15, 9 and 4, at the doses of 250, 500 and 1000 mg/kg bodyweight, respectively, as compared with the mean parasite count of 23.25 in the negative control group. Chloroquine administration of 5 mg/kg chloroquine reduced the mean parasitedensity to 0.1. The dose of extract administered also affected the survival period of the mice. Mean survival time in the distilled water treated group was 10 days. Mice administered with 250, 500 and 1000 mg extract/kg body weight survived for 28, 25 and 24 days respectively. Chloroquine administered mice had survival of 28 days after administration similar to the mice treated with 250mg extract/kg body weight (Table 4).

Prophylactic Activity of the Ethyl Acetate Fraction of the Stem Bark of *U. togoensis*

There was a dose dependent prophylactic activity at the doses employed resulting in a significant reduction in extract treated group when compared to the control. Average percentage chemosuppression of 31.4, 58.2 and 73.1% was respectively recorded for the corresponding doses of extract (250, 500 and 1000 mg/kg/day). Percentage chemosuppression exerted by the standard drug (pyrimethamine 1.2mg/kg/day) was 99.2% (Table 5).

Table 1: Phytochemical Constituents present in the Fraction

Phytochemical constituent	
Carbohydrates	+
Glycosides	+
Anthraquinones	-
Steroids/triterpenes	+
Cardiac glycosides	+
Saponins	++
Tanins	+
Flavonoids	+
Alkaloids	+

+ = present - = absent

Table 2: Suppressive Activity of Ethyl Acetate Fraction of *U. togoensis*

Treatment (Oral)	Group	Dose (mg/kg/day)	Average parasitemia	Average percentage chemosuppression (%)
Distilled water	1	0.2ml	30.83 ± 1.90	
EAF	2	250	18.50 ± 4.93*	39.1
EAF	3	500	14.00 ± 2.87*	53.9
EAF4		1000	8.30 ± 1.55*	72.7
Chloroquine	5	5	0.67 ± 0.33*	97.8

Values were presented as mean ± SEM, n=6, values were significantly different between test and control group at *p<0.05, EAF = Ethyl Acetate Fraction

Table 3: Curative Activity of Ethyl Acetate Fraction of *U. togoensis*

Treatment (Oral)	Group	Dose (mg/kg/day)	Average parasitemia	Average percentage chemosuppression (%)
Distilled water	1	0.2ml	23.25±1.78	
EAF 2		250	15.13±1.62*	34.9
EAF 3		500	9.35±3.09*	59.8
EAF4		1000	4.15±0.49*	82.2
Chloroquine5		5	0.10±0.08*	99.6

Values were presented as mean ± SEM, n=6, values were significantly different between test and control group at *p<0.05, EAF = Ethyl Acetate Fraction

Table 4: Mean Survival Time of mice

Treatment (Oral)	Group	Dose (mg/kg/day)	Mean survival time (days)
Distilled water1		0.2ml	10.00±3.73
EAF 2		250	28.00±0.00
EAF3		500	25.30±0.00
EAF4		1000	24.70±3.83
Chloroquine5		5	28.00±0.00

Values were presented as mean ± SEM, n=6, values were significantly different between test and control group at *p<0.05, EAF = Ethyl Acetate Fraction

Table 5: Prophylactic Activity of Ethyl Acetate Fraction of *U. togoensis*

Treatment (Oral)	Group	Dose (mg/kg)	Average parasitaemia	Average percentage chemosuppression (%)
Distilled water	1	0.2ml	21.37±1.35	-
EAF	2	250	14.65±0.99	31.4
EAF	3		8.94±2.17	58.2
EAF	4	1000	5.75±1.25	73.1
Pyrimethamine5		1.2	0.18±0.12	99.2

Values were presented as mean ± SEM, n=6, values were significantly different between test and control group at *p<0.05, EAF = Ethyl Acetate Fraction

DISCUSSION

The *in vivo* antiplasmodial activity and acute toxicity of the ethyl acetate fraction of *Uapacatogoensis* stem bark extract was investigated. Acute toxicity studies of the ethyl acetate fraction were carried out to determine the safety of the fraction. The oral median lethal dose (LD₅₀) of the fraction was greater than 5000 mg/kg body weight. This suggests that acute oral administration of the fraction is relatively safe (Lorke, 1983). Rodent models have been shown to produce disease features similar to those of human

plasmodial infection, when infected with *P. berghei* (Thomas *et al.*, 1998). Many studies have employed *P. berghei* in predicting treatment outcome of suspected antimalarial agents, because of its high sensitivity to chloroquine (Agbedahunsi, 2000; Adzu and Salawu 2009). Thus *P. berghei* infected mice was used in this study.

The 4 day suppressive test is a standard test commonly used for antimalarial screening (Peters, 1965).

The ethyl acetate fraction produced significant dose related chemo suppression in all the treated groups with the highest chemo suppression (73%) observed in the group treated with 1000 mg fraction/kg. The chemo-suppression value of the highest dose of the fraction was much higher than that of the lower doses indicating that the fraction has more suppressive activity at higher doses. The fraction also demonstrated significant dose related reduction in parasite count in both established (curative) and residual (repository) infection, comparable to the effect of chloroquine and pyrimethamine, which in this study were used as standard control drugs. For the mean survival time, mice administered with the lowest dose of the fraction (250 mg/kg) survived for 28 days comparable to the number of days to the chloroquine treated mice.

The mechanism by which the ethyl acetate fraction exerts its antiparasmodial action has not been elucidated; however, antiparasmodial effects of natural plant products have been attributed to some of their active phytochemical components (Sofowora, 1980; Ayoola *et al.*, 2008). Some of these phytochemicals such as terpenes and flavonoids have been reported to have antiparasmodial activity (Phillipson and Wright, 1990; Christensen and

Kharazmi, 2001; Go, 2003). Preliminary phytochemical screening of the ethyl acetate fraction of *Uapacatogoensis* stem bark revealed the presence of glycosides, phenols, triterpenes, steroids, alkaloids, tannins, carbohydrates, saponins and flavonoids. The antiparasmodial effect of ethyl acetate fraction of *U. togoensis* may therefore be due to the presence of one or all of the phytochemical components present.

CONCLUSION

The findings from the study suggest that the ethyl acetate fraction of *Uapacatogoensis* possesses antiparasmodial activities, and supports the ethno medical claim of the use of the plant in the management of malaria and fever.

CONFLICT OF INTEREST

There is no conflict regarding this work.

CONTRIBUTION OF AUTHORS

OAB designed and carried out the work under the supervision of MBB, CBA AND EJI. HAN was consulted when OAB was writing the paper.

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